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(57) Abstract

Primer sets which amplify conserved regions of specific genes across mammalian species are provided. The methods used to design the primer sets as well as methods of making and using the primer sets are also provided.

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GENE SPECIFIC UNIVERSAL MAMMALIAN SEQUENCE-TAGGED SITES

FIELD OF THE INVENTION

The present invention relates generally to genetic markers and methods of making and using such markers, and more particularly, to primer sets referred to as universal mammalian sequence-tagged site primers, which may be used to amplify conserved regions of specific genes across mammalian species.

BACKGROUND OF THE INVENTION

Over the last several years significant effort has been made to develop genome maps of many species. The subjects of the majority of these projects have been mammals, including human, mouse, rat, ox, sheep, pig, horse, cat, and dog (*e.g.*, Buchanan F.C. et al., *Genomics* 22:397-403 (1994); Dietrich, W. et al., *Genetics* 131:423-447 (1992); Ellegren, H. et al., *Anim. Genet.* 23:133-142 (1992); O'Brien, S.J., *Trends Genet.* 2:137-142 (1986); Serikawa, T. et al., *Genetics* 131:701-721 (1992); Weissbach, J. et al., *Nature* 359:794-801 (1992); Wintero, A.K. et al., *Genomics* 12:281-288 (1991); and Barendse, W. et al., *Nat. Genet.* 6:227-235 (1994)). For non-human mammals, genome maps will lead to important applications including optimized breeding strategies, wherein desirable characteristics are selected and unwanted genes are removed, particularly those genes that lead to genetic disease. Comparisons made between genome maps will also provide a wealth of scientific information (O'Brien, S.J. et al., *Nat. Genet.* 3:103-112 (1993)).

The traditional method for developing gene-specific markers, Southern blotting and cross-species hybridization, is time consuming, labor intensive and limited in flexibility. However despite its many limitations, this method remains the primary method for developing gene-specific markers in most mammalian genome projects. There thus remains a need for a more efficient method. A more efficient method is particularly important for non-human mammalian genome projects wherein scientific resources are often limited.

One potential method is the development of polymerase chain reaction (PCR) primers to conserved regions of specific genes that can be used across species. Although this method has been successfully used for the study of a number of individual genes, it has not been applied on a genome-wide basis for the purpose of map development.

It would thus be desirable to provide genetic markers which may be used to detect specific mammalian genes. It would further be desirable to provide genetic markers which may be used to develop mammalian genome maps. It would further

be desirable to provide genetic markers which may be used across mammalian species. It would also be desirable to provide a method for designing and producing genetic markers based on conserved regions of genes.

SUMMARY OF THE INVENTION

Primer sets which may be used to amplify conserved regions of specific genes, including eleven preferred primer sets that have been shown to amplify specific canine genes, are provided. The methods used to design the primer sets as well as methods of making and using the primer sets for example with a polymerase chain reaction (PCR), are also provided. Such genetic markers based on PCR primers are often called sequence-tagged sites (STSs) or sequence-tagged site primers (Olsen, M. et al., *Science* 245:1434-1435 (1989)). Because the primer sets of the present invention may be used to locate genes across mammalian species, such primer sets are referred to herein as universal mammalian sequence-tagged site (UM-STS) primers.

Additional objects, advantages, and features of the present invention will become apparent from the following description and claims taken in conjunction with the accompanying drawings.

BRIEF DESCRIPTION OF THE DRAWINGS

The various advantages of the present invention will become apparent to one skilled in the art by reading the following specification and claims and by referencing the following drawings in which:

Figure 1 is a photograph of a gel showing the amplification of several canine gene segments using the UM-STS primers of the present invention;

Figure 2 shows the lineup of several canine gene sequences with homologous non-canine mammalian genes;

Figure 3 is a photograph of a gel showing the amplification of a portion of the *FES* proto-oncogene from several mammalian DNAs using the UM-STS primers of the present invention; and

Figure 4 shows sequence comparisons of a portion of the *FES* proto-oncogen from several mammalian DNA.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

The universal mammalian sequence-tagged site primer sets of the present invention are set forth in Tables 1 and 1A. The methods used to design the primer sets as well as methods of making and using the primer sets are also provided.

The primer sets of the present invention referred to herein as UM-STS primers, may be used to amplify mammalian genome regions of interest, isolate clones from mammalian genomic and cDNA libraries and perform cross-species genome comparisons. The UM-STS primers of the present invention will also be useful for developing additional genetic markers within various genomes. For example, a microsatellite repeat has been found within the retinoblastoma (*RB1*) loci disclosed herein. Single site variability may also be found directly in at least some of the amplified products by using one of a number of techniques developed for scanning for variability, such as the single-strand conformation polymorphism technique. For example, this method has been used to find two polymorphic sites in a study of the canine *ALAS2* gene in a PCR product of a size similar to those set forth herein (Boyer, G. et al., *Anim. Genet.* 26:206-207 (1995)). If the frequency of single site polymorphic variability for other mammals is as high as that estimated for humans (roughly one in 200 to 400 nucleotides), then a significant portion of UM-STSs will have these sites. Each species will be screened individually for genetic variability using UM-STS primers.

An example of the usefulness of cross-species genome comparisons is given by the case of Waardenburg syndrome. Direction to the location of one of the human Waardenburg syndrome genes, well known for causing a syndromic hearing loss, was first found from comparative mapping with the mouse (Asher, J.H.J. et al., *J. Med. Genet.* 27:618-626 (1991)). The map locations in the mouse suggested possible locations of the human disease gene, one of which eventually was proven correct (Morrell, R. et al., *Hum. Mol. Genet.* 156:53-59 (1993)). Because the identity of the gene in the mouse was not known at the time, this approach might more properly be called a 'candidate linkage' approach. UM-STSs will thus be useful not only for rapidly producing genetic maps but the candidate linkage approach may also be applied to more species.

Very little is known about the location of genes within the canine genome. The development of UM-STSs will help to rapidly identify the location of linkage groups on specific canine chromosomes. The assignment of conserved syntenies will allow candidate linkages to be tested in the canine genome. The assignment of the proposed anchor loci (O'Brien, S.J. et al., *Nat. Genet.* 3:103-112 (1993)) as defined by UM-STSs to specific chromosomes may be accomplished by the somatic cell hybrid, flow sorted chromosome, and fluorescent in situ hybridization (FISH)

methodologies. Other methods known to those skilled in the art, such as assignment by use of linkage to previously mapped loci, are also possible.

SPECIFIC EXAMPLE 1

The following example further describes the UM-STS primers of present invention as well as methods of designing, making and using such primers.

Materials and Methods

DNA Isolation - DNA from dog, human, pigtail macaque, horse, pig, rat and mouse were isolated from various tissues by standard phenol-chloroform extraction methods (Sambrook, J. et al., Molecular Cloning. A Laboratory Manual. (2nd ed.) Cold Springs Harbor: Cold Springs Harbor Laboratory Press (1989)). Goat DNA was supplied by Dr. Karen Friderici, Michigan State University. DNA was purified by standard methods from a canine liver cDNA library (Clontech) and from a canine genomic DNA library (Clontech) after growing 1 x 10^6 phage in *E. coli* strain LE392 (Murray, N.E. et al., *Mol. Gen. Genet.*156:53-59 (1977)) in liquid culture (Sambrook, J. et al., Molecular Cloning. A Laboratory Manual. (2nd ed.) Cold Springs Harbor: Cold Springs Harbor Laboratory Press (1989)).

Design of PCR Primers - Primers were designed to genes where the intron-exon structure was known in at least one species and where the nucleotide sequence was known in at least two species (the index species) that were not closely related. Tandemly duplicated genes known to have undergone gene conversion in any species were avoided. Primers were generally designed so that the amplified product contained an intron. The human gene nomenclature system (ISGN, *Cytogenet. Cell Genet.* 46:11-28 (1987)) was followed for naming the canine genes. An example of the loci used and their protein products are: *CFTR*, cystic fibrosis transmembrane regulator; *COL10A1*, type X collagen, alpha 1 chain; *CSF1R*, colony stimulating factor 1 receptor; *CYP1A1*, cytochrome P-450 1, alpha 1; *DCN1*, decorin; *FES*, c-fes (feline sarcoma) proto-oncogene; *GHR*, growth hormone receptor; *GLB1*, beta galactosidase; *PKLR*, pyruvate kinase - liver, RBC form; *PVALB*, parvalbumin; and *RB1*, retinoblastoma protein. The Genbank Accession numbers or reference for the sequence of the two index species for each locus are as follows: *CFTR*, M55129, M60493; *COL10A1*, X65120, X65121; *CSF1R*, X14720, K01643; *CYP1A1*, (Uchida, T. et al., *Mol. Pharmacol.* 38:644-351 (1990)), X04300; *DCN1*, L01125, Z12298; *FES*, X06292, J02088; *GHR*, Z11802, J04811; *GLB1*, S59584, M57734; *PVALB*, X63578, M15452; *PKLR*, S59798, M17088; and *RB1*, L11910, M26391.

Primers were designed to highly conserved nucleotide sequences contained within coding regions. Additional considerations taken into account were: degeneracy of underlying codons (Li, W.-H. et al., Fundamentals of Molecular Evolution. Sunderland, MA: Sinauer Associates, Inc. (1987)), overall amino acid mutability of the primer region (Collins, D.W. et al., *Genomics* 20:386-396 (1994)), placement of the 3′ end of the primer with respect to amino acid mutability, as well as standard design practices for primers known in the art such as avoidance of primer-dimers. Conservation of amino acids within multigene families was also taken into account, when possible. Where unavoidable mismatches occurred between the two index species, the primer sequence was designed to match one of the two which was then designated the primary index species. GC-rich genes were avoided due to amplification difficulties, even with exactly matching primers. Primers were twenty bp in length on average. Each primer in a pair was adjusted to be of approximately the same annealing temperature (Breslauer, K. et al., *PNAS (USA)* 83:3746-3750 (1986)). All sets of primer pairs were designed to have approximately the same annealing temperature in anticipation of performing multiplex amplifications. It was not always possible to follow every rule for every gene, given the actual circumstances; however, the majority of the rules were generally applicable.

PCR Amplifications - Correct design and syntheses of the primers were examined by amplifying the DNA from the primary index species. Standard buffer, nucleotide, and primer concentrations were 50 mM Tris-HCl (pH 8.3 at room temperature), 50 mM KCl, 1.5 mM $MgCl_2$, 200 μM dNTPs, 0.1 μg of each primer, and 0.5 - 1.0 μg of target DNA in a 25 μl reaction. Reactions were routinely boiled for three min prior to the addition of 2.0 U of Taq DNA polymerase. Optimal cycling conditions for the amplification of canine genomic DNA were usually found by testing one of several sets of conditions in general use in the lab (see Table 2). Occasionally it was necessary to use "hot-start" conditions (Bassam, B.J. et al., *Biotechniques* 14:30-34 (1993)) in order to get stronger, cleaner amplifications. The presence of an amplification product was determined by electrophoresis of a portion of the reaction on a 1% agarose 1X TBE gel (90 mM Tris, pH 8.3, 90 mM sodium borate, 2.5 mM EDTA) followed by staining with ethidium bromide.

DNA Sequence Analysis - The identity of each amplified canine gene was confirmed by 'single pass' direct sequencing of PCR products using Sequence or Taq DNA polymerase (United States Biochemical Corp., Cleveland). The PCR products were gel purified with Qiaex (Qiagen Corp., Chatsworth, CA) or by elution

from polyacrylamide gel slices (Bergenhem, N.C.H. et al., *Biochem. Genet.* 30:279-287 (1990)) prior to their use in the sequencing reactions. The canine sequences were aligned visually with the sequences of the other species used to design the PCR primers in order to verify the degree of sequence identity.

Results

The primer sets for the various UM-STSs reported here are given in Tables 1, 1A and 2 and efficient amplification conditions for the eleven preferred UM-STSs are given in Table 3. It will be appreciated by those skilled in the art that the conditions may be optimized further (*e.g.*, reduction in the time in each cycle). However, the conditions set forth herein were found to work effectively while minimizing the number of conditions that had to be examined.

A representative gel showing amplification of the canine target DNA along with the human target DNA is shown in Figure 1. In Figure 1 the various lanes were amplified with the following gene-specific primer sets of the present invention: lanes 1-4, *GHR*, lanes 5-8, *COL10A1*, and lanes 9-12, *DCN1*. Lane 13 contained a mixture of DNA size markers; lambda bacteriophage DNA cut with the restriction endonuclease BstE II and the plasmid pSK- (Stratagene) cut with Msp I. Lanes 1, 5, and 9 contain PCR products amplified from human genomic DNA. Lanes 2, 6, and 10 contain PCR products amplified from canine genomic DNA. Lanes 3, 7, and 11 contain PCR products amplified from DNA purified from a canine genomic library contained in a lambda phage vector. Lanes 4, 8, and 12 contain PCR products amplified from a canine liver cDNA library. The human target serves as a positive control for the amplification system because these primers were designed to exactly match the human sequence. The ability to quickly screen genomic and cDNA libraries for the presence of sequences is also demonstrated in Figure 1. The genomic clones for *GHR*, *COL10A1*, and *DCN1* (a very faint signal, stronger on other gels [data not shown]) are present in this particular canine genomic library. The presence of a decorin cDNA clone (encoded by the *DCN1* locus) in the canine liver cDNA library is shown by the presence of the 122 bp band; cDNA clones for *GHR* and *COL10A1* are not present. The *DCN1* PCR product from the cDNA library was sequenced and its identity confirmed (see Figure 2). The human and canine genomic bands have different sizes for *GHR* and *DCN1* because of the intron size differences. The size for the *COL10A1* PCR product is the same between the species because this the only perferred UM-STS set forth herein in which an intron was not spanned. Although the PCR product bands in Figure 1 are unique, some

UM-STS-species combinations contained one to several non-specific amplification products. However, it is almost always possible to deduce the correct band based upon staining intensity and the similarity in size compared to the band of the primary index species.

The amplified products for all of the canine loci (see Table 2) were sequenced to confirm their identity and the results are shown in Figure 2. The locations of PCR primers are underlined, although not all PCR primer sites are shown. Some of the lineups show intron sequence whereas others simply identify the location of the introns. The degree of identity between the canine and index species sequences for each locus is within the range generally accepted (approximately 70 to 100%) as indicating homology for mammalian species (Li, W.-H. et al., Fundamentals of Molecular Evolution. Sunderland, MA: Sinauer Associates, Inc. (1987)). These results support the hypotheses that the canine PCR products are homologous to the respective index species' genes. The canine *COL10A1* sequence matched the human and mouse sequences to a similar extent (data not shown). The sequences for *PKLR* and *CYP1A1* exactly matched previously published canine coding sequences (Whitney, K.M. et al., *Exp. Hematol.* 22:866-874 (1994); and Uchida, T. et al., *Mol. Pharmacol.* 38:644-351 (1990)); the sequence for canine *FES* is shown in Figure 4. Although the majority of the canine sequence for *PVALB* is from an intron, it is believed that the degree of sequence identity from this region is sufficient evidence to confirm that the PCR product is from the correct canine locus. As expected, the canine sequences tend to show greater identity with the human sequences than with the rodent sequences because of the faster evolutionary rate of the rodent genome (Gu, X. et al., *Mol. Phylogen. Evol.* 1:211-214 (1993)). As set forth above, a microsatellite repeat was found within the amplified product itself for *RB1*. Preliminary results show that the *RB1* repeat, $(GA)_{12(avg)}$, has moderate genetic variability within several canine breeds.

The 'universal' utility of these primers was studied on the DNAs from mammals representing several different orders using the primer sets under the reaction conditions that were found to amplify the canine sequences. These reactions are referred to herein as 'Zoo PCRs.' Figure 3 shows a representative experiment. The *FES* proto-oncogene amplified from all of the mammalian species were examined. These DNAs were purified and sequenced directly without subcloning (see Methods and Materials above). In Figure 3, target DNAs for each lane are: 1, human; 2, pigtailed macaque; 3, dog; 4, goat; 5, pig; 6, horse; 7, mouse;

and 8, rat. The mouse DNA was degraded; strong amplification was obtained with another lot (sequence shown in Figure 4). The DNA marker lane (M) contains a 100 bp ladder. The sequences are tabulated in Figure 4. Sequences are from exon 15 and intron 15. Notations for the sequence lineups in Figure 4 are HUM, human; MAC, macaque; CAT, domestic cat; FES, feline sarcoma virus; DOG, dog; COW, ox; GOA, goat; HOR, horse; PIG, pig; RAT, rat; MOU, mouse. The upper two lines for each block of text represent amino acid sequences and the lower lines represent nucleotide sequences. Dots indicate nucleotides in the various species that are identical with that of the human sequence. The human and cat sequences match the published sequences (Alcalay, M. et al., *Oncogene* 5:267-275 (1990); and Roebroek, A.J.M. et al., *J. Virol.* 61:2009-2016 (1987)). The feline sarcoma virus sequence was not determined but is included for comparative proposes. Only a single amino acid interchange was found among these sequences; isoleucine (I) for macaque, cat, and feline sarcoma virus and leucine (L) in all others. Sequence alignments for the intron were done visually.

The degree of sequence identity makes it highly likely that the canine PCR products are homologous with the corresponding index species' genes. The pattern of nucleotide interchange is also what would be expected for homologous genes; members of the same mammalian order share more sequence similarity with one another than with those of other orders.

The data for the Zoo PCRs for the preferred UM-STS primer sets are given in Table 4. Greater than eighty-four percent of the targets, excluding the index and canine species, amplified under the single condition used to amplify the canine sequence. These species represent five different mammalian orders; primates (human and macaque), carnivores (dog), arteriodactyls (goat and pig), perissodactyls (horse), and rodents (mouse and rat). Limited experiments on other members of these orders (*e.g.*, cat and ox) produced similar results (data not shown). Lack of amplification for *DCN1* for one of the arteriodactyls (goat) would be predicted because there are four mismatches between the UM-STS primers and the sequence of the closely related bovine *DCN1* (Day, A.A. et al., *Biochem. J.* 248:801-805 (1987)).

Table 1

Primer sequences for UM-STSs sorted according to chromosomal location.

Seq'd	Locus Name	Gene Product	Human Chromosome Band	Primer 1 Sequence	Primer 2 Sequence
	NRAS	N-RAS Proto-Oncogene	1 p13	ATGACTGAGTACAAACTGGT	CTTCGCCTGTCCTCATGTA
X	PND	Pronatriodilatin	1 p36	GCAGACCTGCTGGATTTCAAG	CAGTCCGCTCTGGGCTCCAAT
	FGR	FGR Protooncogene	1 p36.1	GAGTGGTACTTTGGAAAGATTGG	TAGTGCTGCACCAGCTCCTG
D	CXN40	Connexin 40	1 pter-q12	ATGGGTGACTGGAGCTTCCT	CCCACAATGAAGGCCACCTC
X	PKLR	Pyruvate Kinase - RBC	1 q21	CGCCTCAAGGAGATGATCAA	ATGAGCCCGTCGTCAATGTA
X	AT3	Antithrombin III	1 q23-q25	CTTCTTTGCCAAACTGAACTG	GGGCTGAACTTTGACTTCCA
X	REN	Renin	1 q32	ACACTCCCCGACATCTCTTT	CGCCGATCAAACTCTGTGTA
X	SFTP3	Pulmonary Surfactant Protein 3	2 p11.2	GGAAGTTCCTGGAGCATGAG	CACAGGCCCAGGTGCTTACA
X	SPTBN1	Beta Spectrin (Non-RBC)	2 p21	TCTCAAGACTATGGCAAACA	CTGCCATCTCCCAGAAGAA
X	APOB	Apolipoprotein B	2 p24-p23	TATGATTTTTCCTTTAAATCAAG	GTGCCCTCTAATTTGTACTG
X	IL1A	Interleukin 1 Alpha	2 q13	AGAAGTCAAGATGGCCAAAGT	TGATTCAGAGACAGATGGTC
X	COL3A1	Collagen III Alpha 1	2 q31-q32.3	GGACCAGGAAGTGATGGGAA	ACTTTCTCCTTGACTTCCCT
X	ELN	Elastin	2 q31-qter	GCTGCAGCCGCTAAAGCAG	AGGACACCTCCAAGGCCAG
X	PAX3	Human Paired Domain 2	2 q34-q36	GCCACAAGATCGTGGAGATG	GGTTCTCTCTTTTGTATTCCTC
X	GCG	Glucagon	2 q36-q37	TTCATTGCTTGGCTGGTGAA	GTGTTCATCTCATCAGAGAA
X	PIT1	Pituitary-Specific Transcription Factor 1	3 p11	TTCAGTCAAACAACAATCTG	GCTCCCACTTTTTCATTGTA
X	GNAT-1	Rod Transducin Alpha	3 p22-21.3	CGCAAGAAGTGGATCCACTG	GCAGATGCTGTTGAACAGGT
	RAF1	c-RAF Protooncogene	3 p25	CACATCAACAACCGAGATCA	GCATTGATATCCTCAGTGTG
X	GLB1	B-Galactosidase	3 pter-p21	GAATTCTATACTGGCTGGCT	CATTCCAATAGGCAAAATTGGT
X	GPX1	Glutathione Peroxidase 1	3 q11-q12	GACTACACCCAGATGAACGA	CAGGAACTTCTCAAAGTTCC
X	TF	Transferrin	3 q21	GCTGACAGGGACCAGTATGA	AACAGCAGGTCCTTCCCATG
X	RHO1	Rhodopsin	3 q21-qter	TACATGTTCGTGGTCCACTT	TGGTGGGTGAAGATGTAGAA
X	GLUT2	Glucose Transport-like 2	3 q26.1-q26.3	TGGATGAGTTATGTGAGCAT	GACTTTCCTTTGGTTTCTGG
X	SST	Somatostatin	3 q28	GACTCCCGAGGCTTCCTCTTTG	ATACTGCAGGAGAGAGAAGAA
X	HOX7	Homeobox 7	4 p16.1	AAGTTCCGCCAGAAGCAGTA	ATCTTCAGCTTCTCCAGCTC
X	PDEB	cGMP Phosphodiesterase Beta	4 pter	CTGAAGAGCTACTACACGGA	TGACACTTGTTCATCCACCA
X	ALB	Albumin	4 q11-q13	GGCTGACTGCTGTGCAAAACA	AAGTAAGGATGTCTTCTGGC
X	KIT	c-KIT Protooncogene	4 q12-q13	CCTGTGAAGTGGATGGCACC	GCATCCCAGCAAGTCTTCAT

Table 1 (cont.)

Seq'd	Locus Name	Gene Product	Human Chromosome Band	Primer 1 Sequence	Primer 2 Sequence
X	FGG	Fibrinogen Gamma	4 q28	CAATATAAAGAAGGATTTGGACA	TGACACTTGTTCATCCACCA
	F11	Coagulation Factor XI	4 qter	AATCTTTGTCTCCTTAAAACATC	GGGTATAGGTAAAAAACTGGCA
X	GHR	Growth Hormone Receptor	5 p13.1-p12	CCAGTTCCAGTTCCAAAGAT	TGATTCTTCTGGTCAAGGCA
X	HEXB	Beta Hexosaminidase	5 q13	TTCATTGGTGGAGAAGCTTG	ATCTTTGGAACTCCAGAGTC
	HMGCR	HMG CoA Reductase	5 q13.3-q14	GGATATATGCCCATCCCTGT	GTCATCCCATCTGCAAGGAC
X	IL4	Interleukin 4	5 q23-q31	CTATTAATGGGTCTCACCTCCCAACT	TCAACTCGGTGCACAGAGTCTTGG
X	PDEA	Rod Phosphodiesterase Alpha	5 q31-33	TGCCAAACAGTACTACAACC	TGCAGCTCCAGGTGGTTGA
X	ADRB2	Adrenergic Receptor Beta 2	5 q31-q32	CCCATTCAGATGCACTGGTA	GCAGCCAGCAGAGGGTGAA
X	CSF1R	CSF-1 Receptor	5 q33-q35	TTCCAAAACACGGGGACCTA	CATGCCAGGGCGAGAAGGA
X	TNFA	Tumor Necrosis Factor Alpha	6 p21.3	CTCAGCCTCTTCTCCTTCCT	ATGGGCTCATACCAGGGCTT
X	EDN1	Endothelin 1	6 p24-p23	CCAAAAAGACAAGAAGTGCTG	TGGAACAGTCTTTTCCTTTCTT
X	COL9A1	Collagen IX Alpha 1	6 q12-q14	ATCAGGATTGGCCAAGATGA	GGAATCCTGAAGTCTACATT
	SOD2	Superoxide Dismutase 2	6 q21	AAGTTTAAGGAGAAGCTGAC	TCCCAGTTGATTACATTCCA
X	COL10A1	Collagen Type X Alpha 1	6 q21-q22	ATTCTCTCCAAAGCTTACCC	GCCACTAGGAATCCTGAGAA
X	PLG	Plasminogen	6 q25-q27	CAGCTCCCTGTGATTGAGAA	TAGACACCAGGCTTATTGGG
	ASL	Argininosuccinate Lyase	7 cen-q11.2	ATGACCCATCTCAGCAGGAT	AAGTCCCTTGAGGGTCATCA
X	EPO	Erythropoetin	7 q21	CTCCCTCTGGGCCTCCCAGT	CCATCCTCTTCCAGGCATAGAA
X	CFTR	Cystic Fibrosis Trans. Regulator	7 q31-q32	CTAAGCCATGGCCACAAGCA	CATTGCTTCTATCCTGTGTT
X	TCRB	T-Cell Receptor Beta	7 q35	GACTGTGGCTTCACCTCGG	GATCTCATAGAGGATGGTGG
X	SFTP2	Pulmonary Suractant Protein 2	8 p21	CAGAAACACACGGAGATGGT	GCCATCTTCATGATGTAGCA
X	CA2	Carbonic Anhydrase II	8 q22	CAGTTCCATTTTCAGTGGGG	GGCCAGTCCATCAGGTTGCT
X	TG	Thyroglobulin	8 q24	TTCACCTCAGAGTGCTACTG	GCTTCTCTGTAGCTCATGATCTT
	GGTB2	Beta-1,4-Galactosyltransferase	9 p13	GGGAGGTGTCGCATGATCC	ATTGGCCACTGTGATTTGGGT
X	ALDOB	Aldolase B	9 q21.3-q23	GTGACTGCTGGACATGCCTG	TTTGCAGCCTTGCCACCCC
X	C5	Complement Factor 5	9 q22-q34	TGTGTACGATTCCGGATATTTGA	GCTCCTTCACAGACTTTCTG
X	ABL	ABL Proto-oncogene	9 q34	GAGGACACCATGGAGGTGGA	GTGGATGAAGTTCTTCTTCTC
	IL2R	Interleukin 2 Receptor	10 p15-p14	GCTCTACAGAGAGGTCCTG	GGCAGGAAGTCTCACTCTC
X	RET	RET Proto-Oncogene	10 q11.2	CCCTTCCACATGGATTGAAA	CATCCAGTTAGCATATACAC
	PSAP	Prosaposin	10 q21-q22	AAGTGTGCAAGAAGCTGGT	AAGGAAGGATCCATCACCTC
X	TDT	Terminal Transferase	10 q23-q24	ACCTGGAAGGCCATCCGTGT	CGCCGGAGGTCTCTCTCAAA
X	OAT	Ornithine Aminotranferase	10 q26	CGTGCTCTTCAGGATCCAAA	GCCAGCCATCTACCAGTTCT
X	WT1	Wilms Tumor 1	11 p13	GAGAAACCATACCAGTGTGA	GTTTTACCTGTATGAGTCCT
X	LDHA	Lactate Dehydrogenase A	11 p14-15.5	AACTCCAAGCTGGTCATTAT	GAATCCAGATTGCAACCACT
D	INS	Insulin	11 p15.5	GAGCGCGGCTTCTTCTACAC	GGTAGAGGGAGCAGATGCTGG
X	CD20	CD20	11 q12-q13.1	CTCTTTGCTGCCATTTCTGGAAT	TGGAAGAAGGCAAAGATCAGCAT
X	ROM1	Rod Outer Segment Protein-1	11 q13	CAGAGGACGGGCCACAGAA	GTTAAACACCACAGAGGCCTT
X	APOC3	Apolipoprotein C3	11 q23-qter	CAGGAACAGAGGTGCCATGC	TGCGCCACCTGGGACTCCTG

Table 1 (cont.)

Seq'd	Locus Name	Gene Product	Human Chromosome Band	Primer 1 Sequence	Primer 2 Sequence
X	VWF	von Willebrand's Factor	12 p	CCAGAGCGCATGGAGGCCTG	CTGCACTCCAGCTTGAATCC
X	LDHB	Lactate Dehydrogenase B	12 p12.1-12.2	TTCCTCAGATCGTCAAGTACA	CTGCTGGGATGAATGCCAAG
X	IL6	Interleukin 6	12 p12.2-p12	GCACTGGCAGAAAACAACCT	ATCTGAAACTCCACAAGACC
X	TPI	Triosphosphate Isomerase	12 p13	TATATCGACTTCGCCCGGCA	ATGGCCCACACAGGCTCATA
X	COL2A1	Collagen II Alpha 1	12 q14.3	CTCTGCGACGACATAATCTG	TCTCCAGGTTCTCCTTTCTG
X	DCN1	Decorin	12 q21-q23	GTTGATGCAGCTAGCCTGAA	AAGTGAAGCTCCCTCAGATG
X	IGF1	Insulin-Like Growth Factor 1	12 q22	GGCATCGTGGATGAGTGCTG	CTCCTTCTGTTCCCCTCCTG
X	PLA2	Phospholipase A2	12 q23-qter	GACTACGGCTGCTACTGTG	TTACAGCTGGCCAGTTTCTT
X	RB1	Retinoblastoma 1	13 q14.2	GTTCCAGAAAATAAATCAGATGGT	ACTCATTTCTGCCAGTTTCTG
X	F7	Clotting Factor VII	13 q34	AATGGAGCTCAGTTGTGTGG	CGATGTCGTGGTTGGTGGT
X	CHY	Chymase (Mast Cell)	14 q11.2	GTCCCACCTGGGAGAATGTG	TGGGAGATTCGGGTGAAGAC
	FOS	FOS Proto-Oncogene	14 q24.3	TCTGTGGCCCCATCGCAGAC	ATCTTGCAGGCAGGTCGGTG
X	CKBB	Creatine Kinase Brain	14 q32.3	TGGATCAACGAGGAAGACCA	TTCACACCATCCACCACCAT
X	TCRA	T-Cell Receptor Alpha	14 q34	ACTGTCTGCCTGTTCACCGATTT	GTAACAACTTGGCATCACAGGAAT
X	B2M	Beta-2 Microglobulin	15 q21-q22.2	TTCAGCAAGGACTGGTCTTT	CTGCTTACATGTCTCGATCT
X	CYP1A1	Cytochrome P-450 (AHH)	15 q22-q24	TTGGACCTCTTTGGAGCTGG	TGGTTGATCTGCCACTGGTT
X	PKM	Pyruvate Kinase - Muscle	15 q22-qter	GCCTTCATTCAGACCCAGCA	ATTCCAGACTTAATCATCTCCTT
X	FES	FES Proto-Oncogene	15 q25-qter	GGGGAACTTTGGCGAAGTGTT	TCCATGACGATGTAGATGGG
X	HGBA	Alpha Hemoglobin	16 p13.3	CCCACCACCAAGACCTACTT	CGGTATTTGGAGGTCAGCAC
X	GOT2	Glutamate Oxaloacetate Transaminase 2	16 q21-q22	TTTAAGTTCAGCCGAGATGT	CTTGGTAGGCCATGTCAAA
D	CTRB	Chymotrypsinogen	16 q22.3-q23.2	AACGACATCACCCTGCTGTT	TGCAGGAGGAGACGCCACT
X	APRT	Adenosine PR Transferase	16 q24	GACTCCCGAGGCTTCCTCTTTG	ATACTGCAGGAGAGAGAAGAA
X	TP53	Tumor Protein 53	17 p13.1	TACAAGCAGTCACAGCACAT	TCTTCCAGTGTGATGATGGT
X	GUC2D	Guanylate Cyclase 2D	17 p13.1	AGCTTACCGCATCCACGTGA	TTTGGGGATGGGCTTGTTGA
X	NF1	Neurofibromatosis 1	17 q11.2	ATTCACTCTCTGTGTACTTG	CAAAGCTTCTGTGACTGTTT
	KRT10	Keratin 10	17 q12-q21	ACCTACCGCAGCCTGCTAGA	TGACCTTGGTCCCTTAGATGA
X	PDEG	Rod Phosphodiesterase Gamma	17 q21.1	AGGAAGCACAGCTTCTTCAT	GACATCCCTGGAATGGAAG
X	SCN4A	Skeletal Muscle Sodium Channel	17 q23.1-q25.3	CTCAAGGTGGACATCCTGTACAA	AGCAGCGTCCGGATGCCCTT
X	TS	Thymidylate Synthetase	18 pter-q12	TGCCAGTTCTATGTGGTGAA	AGGTAAATATGTGCATCTCC
	TTR	Transthyretin	18 q11.2-q12.1	GACTGGTATTTGTGTCTGAGG	CAGAGGCAAATGGCTCCCA
	C3	Complement Factor 3	19 p13.3-p13.2	GGAGTGGACTATGTGTACAAGAC	TCAGGCCAGTGCTCCACCCT
X	APOC2	Apolipoprotein C2	19 q13.2	GAATCACTCTACAGTTACTGG	AGCTGCTGTGCTTTTGCTGTA
X	CKMM	Creatine Kinase Muscle	19 q13.2-q13.3	AAGAAGCTGCGGGACAAGGA	CAGCCCACGGTCATGATGAAA
	MX1	MX Anti-Viral Protein	21 q22.3	TGAAGAAGGGTTACATGATTGT	GGCAGGGAACCGTGGCCTT
X	PVALB	Parvalbumin	22 q12-q13.1	ATGTGAAGAAGGTGTTTCACAT	TCTTTGTCTCCAGCAGCCAT
	MAOA	Monoamine Oxidase A	X p11.4-p11.23	AGGAACGGAAGTTTGTAGG	CTCCTTGTAATACATCATGC
X	DYS	Dystrophin	X p21	GTTTCAGGCCAGACCTCTTT	TACCGACCTTCAGGATCAAG

Table 1 (cont.)

Seq'd	Locus Name	Gene Product	Human Chromosome Band	Primer 1 Sequence	Primer 2 Sequence
X	MNK	Menkes Protein	X q12-q13.3	GGCATGACTTGTAATTCCTG	CATCAAATCCCATGTCTTCTAT
	PGK1	Phosphoglucokinase	X q13	ATTACCTTGCCTGTTGACTT	AAAAGCTTCCCATTCAAATAG
	PLP	Proteolipid Protein	X q21.3-q22.3	TTAGAGTGCTGTGCAAGATG	CCTAGCCATTTTCCCAAACA
X	HPRT	Hypoxanthine PR Transferase	X q26	AGCTTGCTGGTGAAAAGGAC	TTATAGTCAAGGGCATATCC
X	F9	Clotting Factor IX	X q26.3-q27.1	TGGGTGGTAACTGCAGCCCACT	CTACGCACACTCTTCACCCCA
	F8	Clotting Factor VIII	X q28	GATGCACAGATTACTGCTTC	GTAAGCAGAGATTTTACTCCCTG
X	SRY	Sex Determining Region - Y	Y p11.3	AAGCGACCCATGAACGCATT	TTCGGGTATTTCTCTCTGTG

Note: X in Seq'd (sequenced) column indicates PCR product sequenced;
D indicates primers designed to canine sequence from literature references.

Table 1A

Primer sequences for UM-STSs sorted according to chromosomal location.

S[1]	Locus Name	Gene Product	Human C'some	Band	Primer 1 Sequence	Primer 2 Sequence
	VCAM1	Vascular CAM 1	1	p32-p31	CCAGAGAAACCATTTACTGTTG	CCCTTAAGTAATTCAATCTCCT
	GNB1	Guanine-Nucleo. Bind. Protein - Beta	1	pter-p31.2	ATGAGTGAGCTTGACCAGT	GTTTGTGATCTGAGAGAGAG
	CD23	CD23	1	q21-q23	TTCCAACGGAAGTGCTACTA	GCATCATCACGCAGTCCTC
	AGT	Angiotensinogen	1	q40-q42	GCAGGACCTGCTCGCCCAG	TGTTGGGTAGACTCTGTGGGCT
X	SAG	S-antigen	2	q37	AGTGTATGTCACTCTGACC	TGTTGCTCCCCAGCTTCTT
X	CNCG1	CGMP-Regulated Channel	4	p14-q13	GACCCAGAGAAGAAAAAGAA	GTCCAGTTGTACATAACAG
	EGF	Epidermal Growth Factor	4	q25-q27	GTGCTTGAGAGAGATGGGAA	TACCCAGGAAAGCAATCACA
	GRL	Glucocorticoid Receptor	5	q31-q32	CCCAAACTCTGCCTGGTGTG	AAGACATTTTCGATAGCGGCA
X	RDS	Peripherin/RDS	6	p	TTTGACCAGAAGAAGCGGGT	TTGCTGATCCACTGAATCTC
X	GCAP	Guanylate Cyclase Activating Protein	6	p21.1	GATTGACGTCAACGGGGATG	AGGAGCATCTGGTCCTTCTG
	GCK	Glucokinase	7	p15 (p13)	GAGCAGATCCTGGCAGAGTT	GTCATGGCGTCCTCGGGGAT
X	GNGT1	Transducin-gamma	7	q21.3	GTGTCCAAATGTTGTGAAGA	TGAAATCACACAGCCTCCTT
	PLAT	Plasminogen Activator - Tissue	8	p12-q11.2	AAGCAACCGGGTGGAATATTG	GAGAAGTACAGGGCCTGCTG
	MTS1	Multiple Tumor Suppressor 1	9	p21-22	CAGGTCATGATGATGGCCAG	CGGCATCTATGCGGGCATG
X	TYRP1	Tyrosinase Related Protein 1	9	p23	GCAAAGCGCACAACTCACC	GTAATAGTGTGTCCAAACAA
	ALDH1	Aldehyde Dehydrogenase 1	9	q21	AAACCAGCAGAGCAAACTCC	CATGTGAGAAGAAATGGCTG
	VIM	Vimentin	10	p13	GCTGCCAACCGGAACAATGA	CATTGAGCAGGTCTTGGTATTC
	IRBP	Interstitial Retinal Binding Protein	10	q11.2	GAACGCTATGGCTCCAAGAA	TTGTGCTGGAGCATCTCCT
	PLAU	Plasminogen Activator - Urokinase	10	q24-qter	ACCACCATCGAGAACCAGCC	GGCAGGCAGATGGTCTGTAT
	GOT1	Glutamate Oxaloacetate Transam. 1	10	q24.1-q25.1	GCACTTCGAATTGGAGCTGA	CCTCTCTTCTCTGCATCCCA
	TYR	Tyrosinase	11	q14-21	GAACAAGCGAGTCGGATCTG	GCTCTGATACAAGCTGTGGT
	IGHE	Immunoglobulin E	14	q32.33	ACCTGTCTGGTGGTGGACCT	TCCTCAGGCATGAAGTTCTGGAT
X	AGC1	Aggrecan	15	q25-q26.2	ATGGACATGTGCAGTGCCGGCT	CCTCGGGCTTCACCCTCAGT
	IL4R	Interleukin 4 Receptor	16	p11.2-12.1	GTCCTCCAGCATGGGGCAG	GCTTGAGAAGGCCTTGTAACC
X	MC1R	Melanocortin-1 Receptor	16	q24	AACCTGCACTCACCCATGTA	AGGAAGAGGTTGAAGTTCTTGA
	BRCA1	Breast Cancer 1	17	q21	CTGTGTGAGAGAAAAGAATGG	TTCTTCCTTTTGTTCACATTCAAA
	TK1	Thymidine Kinase 1	17	q23.2-q25.3	GCACTGGATGGGACCTTCCA	ACCGCCGTCAGCTTCACCAC
X	RCV1	Recoverin	17		TGGTACCAGTCCTTCCTGA	CATGACGATCTCCAGCACTT
	FECH	Ferrochelatase	18	q21.3	ATAGCATTTACCAGTGACCA	AACAATGGATTTCCATTAAGAGA
	PRNP	Prion Protein	20	pter-p12	ACCACCAAGGGGGAGACCT	CCCACTATCAGGAAGATGA
	AGT1	Agouti Homologue	20	q11.2	GGATGTCACCCGCCTACT	TTGGATTTCTTGTTCAGTG
	ADA	Adenosine Deaminase	20	q13.11	ATCCTGTGCTGCATGCGCC	ATGGTCTCATCTCCAGCCA
	SOD1	Superoxide Dismutase I	21	q22.1	TGTACCAGTGCAGGTCCTCA	CCCAGGTCTCCAACATGCCT
	PDGFB	Platelet Derived Growth Factor B	22	q12.3-q13.1	CGCTGCTCCGGCTGCTGTAA	AGGGTCACCGTGGCCTTCTT

[1] An X in this column indicates that the PCR product has been confirmed to be the correct locus in the canine genome by sequence analysis

Table 2

Primer Sets for eleven preferred UM-STSs.

Locus	Index Species[a]	Primer 1[b]	Primer 2	Primer 1 Name[c]	Primer 2 Name	P1 A.A.[d]	P2 A.A.	Human Chromosome
CFTR	Human	CTAAGCCATGGCCACAAGCA	CATTGCTTCTATCCTGTGTTC	HCFTREX22D	HCFTREX23U	1346	1407	7q31-q32
	Mouse	.....T.....	...C.....					
COL10A1	Human	ATTCTCTCCAAAGCTTACCC	GCCACTAGGAATCCTGAGAA	HCOL10A1EX2D	HCOL10A1EX2U	505	693	6q21-q22
	Mouse	.....T.....	.....					
CSF1R	Human	TTCCAAAACACGGGGACCTA	CATGCCAGGGCGAGAAGGA	HCSF1REX3D	HCSF1REX4U	76	161	5q33-q35
	FeSV	.....	.G.....					
CYP1A1	Dog	TTGGACCTCTTTGGAGCTGG	TGGTTGATCTGCCACTGGTT	DCYP1A1EX3D	DCYP1A1EX5U	319	417	7q31
	Human	.....	.....					
DCN1	Human	GTTGATGCAGCTAGCCTGAA	AAGTGAAGCTCCCTCAGATG	HDCN1EX6D	HDCN1EX7U	207	247	12q21-q23
	Rat	.....C.....	.....G.....					
FES	Human	GGGGAACTTTGGCGAAGTGTT	TCCATGACGATGTAGATGGG	HFESEX14D	HFESEX15U	573	641	15q25-qter
	FeSV	.....A.....	.....					
GHR	Human	CCAGTTCCAGTTCCAAAGAT	TGATTCTTCTGGTCAAGGCA	HGHREX9D	HGHREX10U	301	432	5p13.1-p12
	Rat	.....A.....	A.....					
GLB1	Human	GAATTCTATACTGGCTGGCT	CATTCCAATAGGCAAAATTGGT	HGLB1EX8D	HGLB1EX9U	268	319	3pter-p21
	Mouse	..G.....	.....					
PKLR	Human	CGCCTCAAGGAGATGATCAA	ATGAGCCCGTCGTCAATGTA	HPKLREX4D	HPKLREX6U	72	193	1q21
	Rat	.....	.....					
PVALB	Human	ATGTGAAGAAGGTGTTTCACAT	TCTTTGTCTCCAGCAGCCAT	HPVALBEX3D	HPVALBEX4U	43	93	22q12-q13.1
	Rat	.....C.....	..C.....					
RB1	Human	GTTCCAGAAAATAAATCAGATGGT	ACTCATTTCTGCCAGTTTCTG	HRB1EX25D	HRB1EX26U	844	905	13q14.2
	Mouse	.....C.....	.....					

[a] Primary index species on top
[b] Dots indicate identical nucleotides
[c] First letter, primary index species; next letters; EX + number = exon number; D = down, U = up
[d] Amino acid over which 5' nucleotide of primer lies

Table 3

Amplification conditions for eleven preferred canine UM-STSs.

Locus	Temperatures (°C)	Times (min)	Size of PCR Product (bp) Human	Dog
CFTR	95, 57, 72	0.5, 1.5, 4	700	1000
COL10A1	94, 57, 72 (hs)[a]	1, 2, 3	384	384
CSF1R	94, 59, 72	1, 2, 3	730	730
CYP1A1	95, 57, 72	0.5, 1.5, 4	700	600
DCN1	94, 57, 72	1, 2, 3	1422	2000
FES	94, 57, 72	0.5, 1, 1.5	484	500
GHR	94, 57, 72	1, 2, 3	765	800
GLB1	94, 57, 72	1, 2, 3	238	240
PKLR	94, 59, 72	1, 2, 3	600	630
PVALB	94, 57, 72(hs)	0.5, 1.5, 4	1400	1300
RB1	94, 59, 72	1, 2, 3	695	1300

[a] hs indicates 'hot start' used.

Table 4

Locus	Human	Macaque	Dog	Goat	Pig	Horse	Mouse	Rat
CFTR	+[a]	+	+	-	+	+	+	+
COL10A1	+	+	+	-	+	+	+	+
CSF1R	+	+	+	+	-	-	+	+
CYP1A1	+	+	+	+	+	+	+	+
DCN1	+	+	+	-	+	+	+	+
FES	+	+	+	+	+	+	+	+
GHR	+	+	+	+	+	+	+	+
GLB1	+	+	+	+	+	+	+	+
PKLR	+	+	+	+	+	-	+	+
PVALB	+	+	+	+	+	+	-	-
RB1	+	+	+	-	+	+	+	+

+, amplification; -, no amplification

[a] Bold symbols indicate index

The foregoing discussion discloses and describes merely exemplary embodiments of the present invention. One skilled in the art will readily recognize from such discussion and from the accompanying claims and drawings, that various changes, modifications and variations can be made therein without departing from the spirit and scope of the invention.

All publications referred to herein are expressly incorporated by reference.

WE CLAIM:

1. A primer comprising a polynucleotide, wherein the polynucleotide has a sequence selected from the group consisting of the sequences set forth in Table 1.

2. A primer comprising a polynucleotide, wherein the polynucleotide has a sequence selected from the group consisting of the sequences set forth in Table 1A.

3. A method for amplifying DNA, comprising the step of performing PCR with the DNA and a primer set selected from the group consisting of the primer sets of Table 1.

4. A method for amplifying DNA, comprising the step of performing PCR with the DNA and a primer set selected from the group consisting of the primer sets of Table 1A.

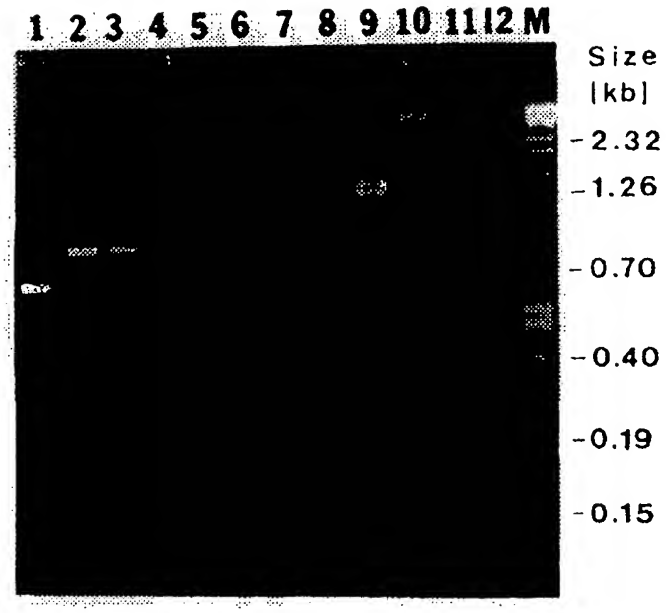
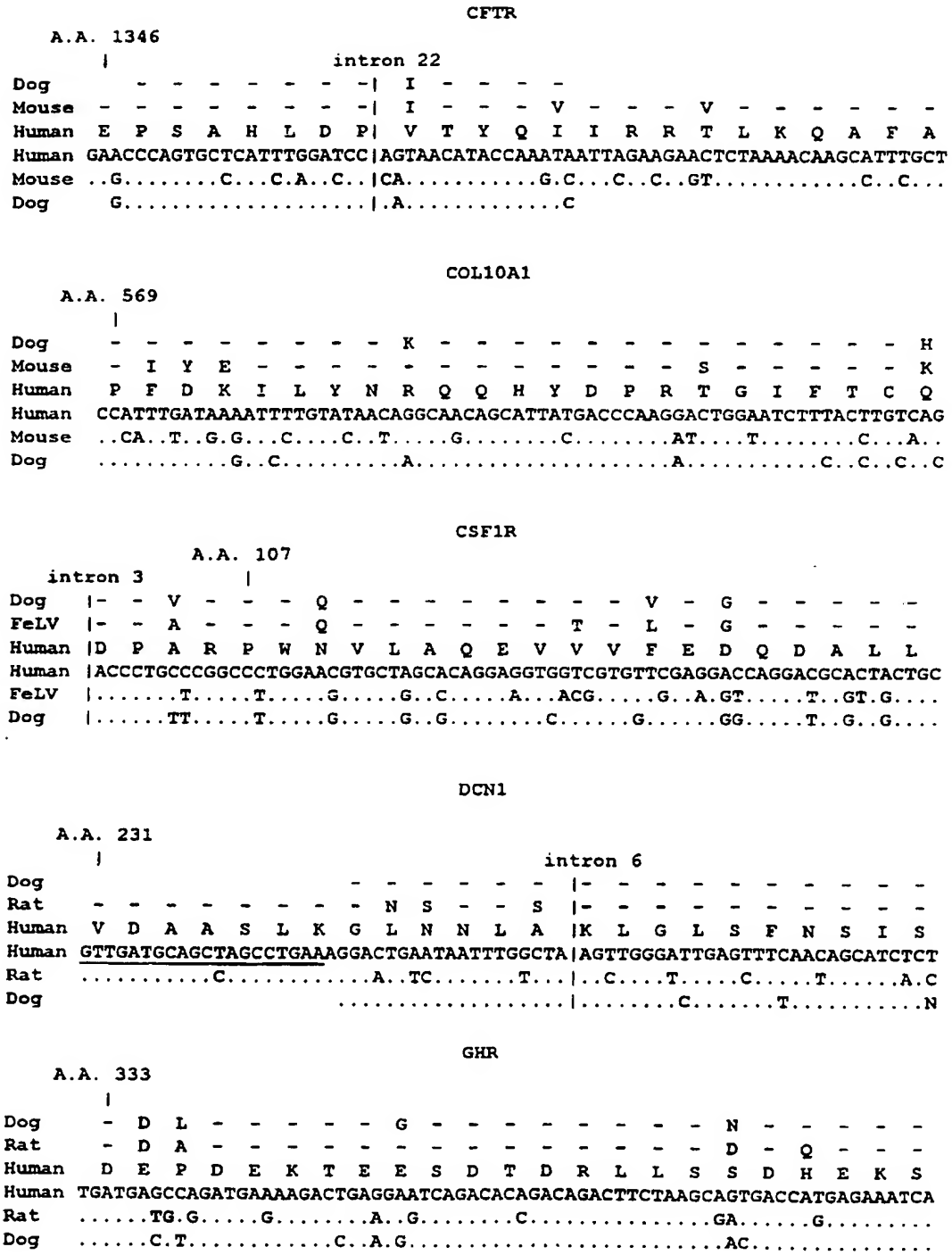


Fig. 1.

Figure 2


CFTR
A.A. 1346
intron 22
Dog - - - - - - - -| I - - - -
Mouse - - - - - - - - -| I - - - - V - - - - V - - - - - - -
Human E P S A H L D P| V T Y Q I I R R T L K Q A F A
Human GAACCCAGTGCTCATTTGGATCC|AGTAACATACCAAATAATTAGAAGAACTCTAAAACAAGCATTTGCT
Mouse ..G.....C...C.A..C..|CA.....G.C...C..C..GT.....C..C...
Dog G.....|.A.....C
COL10A1
A.A. 569
Dog - - - - - - - - - K - - - - - - - - - - - - - - - H
Mouse - I Y E - - - - - - - - - - - - - - S - - - - - - K
Human P F D K I L Y N R Q Q H Y D P R T G I F T C Q
Human CCATTTGATAAAATTTTGTATAACAGGCAACAGCATTATGACCCAAGGACTGGAATCTTTACTTGTCAG
Mouse ..CA..T..G.G...C....C..T.....G.....C.....AT....T.....C...A..
Dog .....G..C.....A.....A.....C..C..C..C
CSF1R
A.A. 107
intron 3
Dog |- - V - - - - Q - - - - - - - - - V - G - - - - - -
FeLV |- - A - - - - Q - - - - - - - - T - L - G - - - - - -
Human |D P A R P W N V L A Q E V V V F E D Q D A L L
Human |ACCCTGCCCGGCCCTGGAACGTGCTAGCACAGGAGGTGGTCGTGTTCGAGGACCAGGACGCACTACTGC
FeLV |.....T.....T.....G.....G..C.....A...ACG.....G..A.GT.....T..GT.G....
Dog |.....TT.....T.....G.....G..G.....C.....G.....GG.....T..G..G....
DCN1
A.A. 231
intron 6
Dog - - - - - - - |- - - - - - - - - - -
Rat - - - - - - - - - N S - - S |- - - - - - - - - - -
Human V D A A S L K G L N N L A |K L G L S F N S I S
Human GTTGATGCAGCTAGCCTGAAAGGACTGAATAATTTGGCTA|AGTTGGGATTGAGTTTCAACAGCATCTCT
Rat .....C.....A..TC.....T...|..C....T.....C.....T.....A.C
Dog .....|.....C.....T.....N
GHR
A.A. 333
Dog - D L - - - - - - G - - - - - - - - - N - - - - - -
Rat - D A - - - - - - - - - - - - - - - - D - Q - - -
Human D E P D E K T E E S D T D R L L S S D H E K S
Human TGATGAGCCAGATGAAAAGACTGAGGAATCAGACACAGACAGACTTCTAAGCAGTGACCATGAGAAATCA
Rat .....TG.G.....G.....A..G.....C.....GA.....G.....
Dog .....C.T.....C..A.G.....AC.....

GLB1

```
   A.A. 268
        |
Dog                                 - - - - - - - - - - V - - - V - - -
Mouse - - - - - - - - - - - - K - - - - - V - - K T L - T
Human E F Y T G W L D H W G Q P H S T I K T E A V A S
Human GAATTCTATACTGGCTGGCTAGATCACTGGGGCCAACCTCACTCCACAATCAAGACCGAAGCAGTGGCTTCC
Mouse ..G.....................C........TA....C..T.....GG.G..A..TA..A..C.....A..
Dog                           .....T........G..A.....A...G.G.....T....TC.........
```

PVALB

```
    A.A. 59
        |
Dog                         -                intron 2                - -
Rat     - - - - - - - -                        (bp)                 S - - -
Human   I  E  E  D  E  L  G                                           F  I  L  K
Human ATCGAGGAGGATGAGCTGGGgtaagctggagg  -  1300  -  tttctcctccagATTCATCCTAAAAG
Rat         ..T.....................a.  -  1500  -  .......-....G.C...T..G..G.
Dog                       .......agactcc.  -  1300  -  .......-.............
```

RB1

```
   A.A. 890
        |                                                          intron 22
Dog     A - - - - - - - - - - - - L - - - - - - - - - - - - - -|
Mouse   - G - - - - - - - - - N V - - - - - - - A - - - - - - -|
Human   G  S  N  P  P  K  P  L  K  K  L  R  F  D  I  E  G  S  D  E  A  D  G  S|
Human GGAAGCAACCCTCCTAAACCACTGAAAAAACTACGCTTTGATATTGAAGGATCAGATGAAGCAGATGGAAG|
Mouse ..CG.......C..C..............CG.G........C..C..G...G.C..............G..|
Dog   .C...................T............TG........C.........................|
```

Figure 2 (cont.)

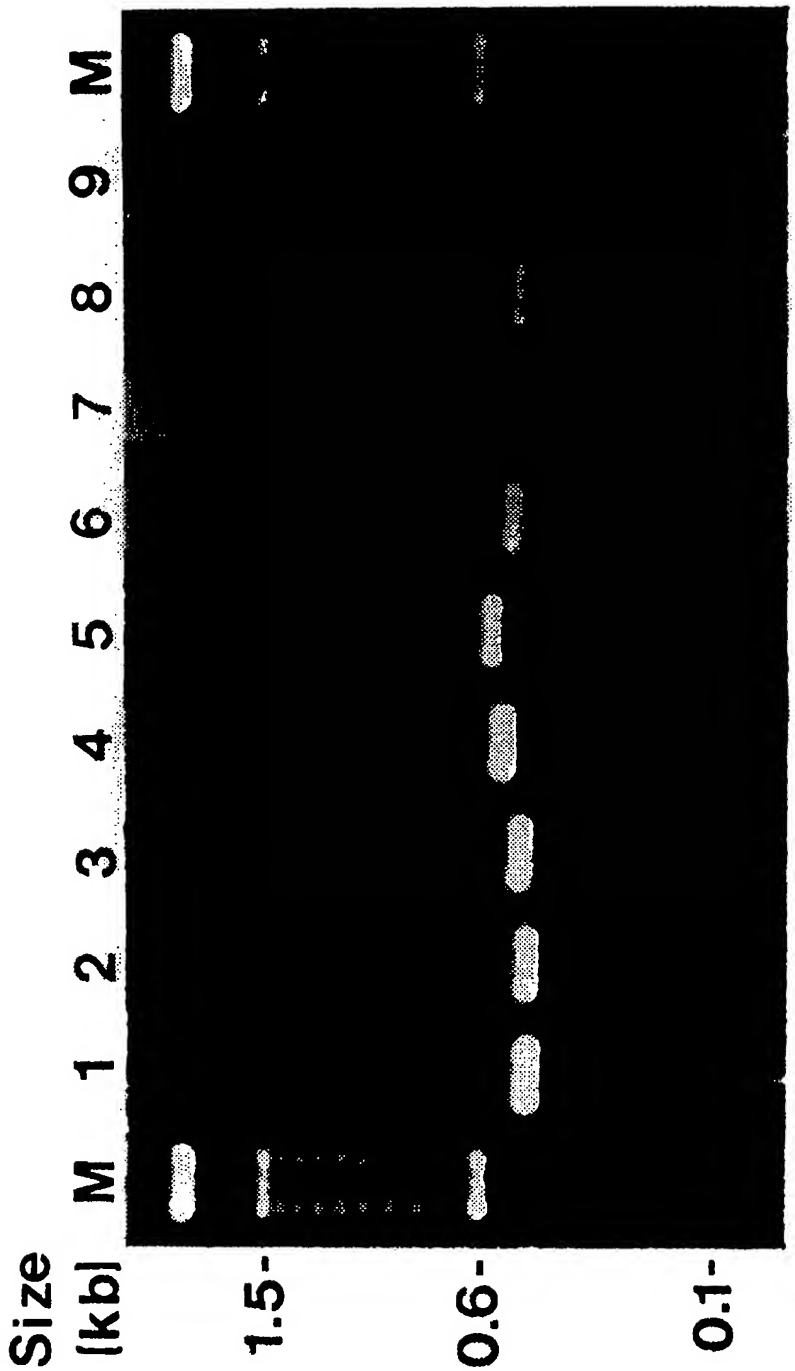


Fig. 2

Figure 4

```
MAC,CAT,FES                                                                I
HUM   A  D  N  T  L  V  A  V  K  S  C  R  E  T  L  P  P  D  L  K
HUM  GCCGACAACACCCTGGTGGCGGTGAAGTCTTGTAGAGAGACGCTCCCACCTGACCTCAAG
MAC  ............T.......A.....................................A.....
CAT  ............T.......C.....A......C.C.....A.............A.....
FES  ............T.......C.....A......C.C.....A.....A......A.....
DOG  ........T..T..............A.....CC.......C..................
COW  ..A.......................A......C.C.....A..................
GOA  ..A.......................A......C.......A..G..C............
PIG  ..A..T....................A.....CC....A.....................
HOR  ..T.......................A.....CC.............C..G.........
RAT  ..A..........C......T.............C.....................N.....
MOU     ..................T.............C......NNN..................
```

```
HUM   A  K  F  L  Q  E  A  R
HUM  GCCAAGTTTCTACAGGAAGCGAG GTGGGTGATAAACTAATGATCACCACGGGTCCCGCAT
MAC  ........................ ..........................................
DOG  ........................ .......C....G...C...--..CA.A.CT..A...
CAT  ............T........A.A ..A....AC...AG..C...--..CATAA.T.....C
FES  ............T........A.A
COW  ............C.............. ..........G.AC.CCC...A.TGTA..C...CATA
GOA  ............G........A.. ..........G.AC.CC...A.TGTA..C...T.C.C
PIG  ............G.............. ............AG..CC....TGTGATAAAAGA.CC
HOR  ..................G..A.. .........C...A...CC....TGGTAT.CTAA.G..
RAT  ............G..NNNN..... ......C.....A.GGGA.CAGT..A..T...TTGTG
MOU  ......................A.. ............A....AT
```

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/02403

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :C07H 21/04; C12P 19/34; C12Q 1/68
US CL : 536/24.31, 24.33; 435/91.2, 6
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 536/24.31, 24.33; 435/91.2, 6

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, DIALOG

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	HUGHES et al. Evolution of the Major Histocompatibility Complex: Independent Origin of Nonclassical Class I Genes in Different Groups of Mammals. Mol. Biol. Evol. 1989, Vol .6, No. 6, pages 559-579, see Abstract.	1-4
Y	CZELUSNIAK et al. Phylogenetic Origins and Adaptive Evolution of Avian and Mammalian Haemoglobin Genes. Nature. 15 July 1982, Vol. 298, pages 297-300, especially page 300.	1, 3
Y	GRAY, M.W. Mitochondrial Genome Diversity and the Evolution of Mitochondrial DNA. Can. J. Biochem. 1982, Vol. 60, pages 157-171, especially page 165.	1-4

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
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"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 12 APRIL 1997	Date of mailing of the international search report 30 APR 1997
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer PHUONG BUI Telephone No. (703) 308-0196

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/02403

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	VENTA et al. Organization of the Mouse and Human Carbonic Anhydrase II Genes. Ann. N.Y. Acad. Sci. 1984, Vol. 429, pages 309-323, especially Abstract and page 319.	1, 3
Y	VENTA et al. Comparison of the 5' Regions of Human and Mouse Carbonic Anhydrase II Genes and Identification of Possible Regulatory Elements. Biochim. Biophys. Acta. 1985, Vol. 826, pages 195-201, see entire article.	1, 3
Y	WICHMAN et al. In Search of Retrotransposons: Exploring the Potential of the PCR. Biotechniques. 1992, Vol. 13, No. 2, pages 258-265, see Abstract.	1-4
Y	MAZZARELLA et al. Conserved Sequence-Tagged Sites: A Phylogenetic Approach to Genome Mapping. Proc. Natl. Acad. Sci. USA. May 1992, Vol. 89, pages 3681-3685, see Abstract.	1-4
Y	HINO et al. Universal Mapping Probes and the Origin of Human Chromosome 3. Proc. Natl. Acad. Sci. USA. January 1993, Vol. 90, pages 730-734, see Abstract.	1-4
Y	SKAGGS et al. The Deduced Amino Acid Sequence of Human Carbonic Anhydrase-Related Protein (CARP) is 98% Identical to the Mouse Homologue. Gene. 1993, Vol. 126, pages 291-292, see entire article.	1, 3
Y	MERRYMAN et al. Characterization of a New, Potent, Immunopathogenic Epitope in S-Antigen that Elicits T Cells Expressing Vβ8 and Vα2-Like Genes. J. Immunol. 01 January 1991, Vol. 146, No. 1, pages 75-80, especially pages 77 and 79.	2, 4
Y	ITAKURA et al. Expression in *Escherichia coli* of a Chemically Synthesized Gene for the Hormone Somatostatin. Science. 09 December 1977, Vol. 198, No. 4321, pages 1056-1063, see Abstract and pages 1056-1058.	1, 3

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